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Comparison of toxicity values across zebrafish early life stages and mammalian studies: Implications for chemical testing

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Abstract

With the high cost and slow pace of toxicity testing in mammals, the vertebrate zebrafish has become a tractable model organism for high throughput toxicity testing. We present here a meta-analysis of 600 chemicals tested for toxicity in zebrafish embryos and larvae. Nineteen aggregated and 57 individual toxicity endpoints were recorded from published studies yielding 2695 unique data points. These data points were compared to lethality and reproductive toxicology endpoints analyzed in rodents and rabbits and to exposure values for humans. We show that although many zebrafish endpoints did not correlate to rodent or rabbit acute toxicity data, zebrafish could be used to accurately predict relative acute toxicity through the rat inhalation, rabbit dermal, and rat oral exposure routes. Ranking of the chemicals based on toxicity and teratogenicity in zebrafish, as well as human exposure levels, revealed that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), benzo(a)pyrene, and chlorpyrifos ranked in the top nine of all chemicals for these three categories, and as such should be considered high priority chemicals for testing in higher vertebrates.

Keywords

Meta-analysis; Toxicity; Teratogen; Zebrafish; Human exposure

1. Introduction

Rodent and rabbit toxicity testing has been the standard for assessing acute toxicity by the United States government since the 1950s. However, the process is costly and time consuming which has led to a backlog in chemical testing [1]. In addition, up to a thousand

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Conflict of interest

The authors declare that there are no conflicts of interest.

new chemicals are introduced to the market yearly [2]. Thus, new screening methods are needed to keep pace with the development of new chemicals and protect both human and environmental health. Zebrafish, *Danio rerio*, have emerged as a viable vertebrate organism for chemical risk testing with over 1490 papers published since the initial paper exploring the effect of zinc sulphate during different stages of zebrafish development in 1965 [3]. Zebrafish are a cost-effective model for chemical toxicity screening due to their high fecundity, rapid embryonic development, and high homology to mammalian species [4]. In addition, they provide a whole animal model advantage over cell lines allowing for metabolism and systemic interactions to mimic the processes in the human body. Because of its small size, the zebrafish embryo or larvae has been used successfully in high throughput toxicity screening. The embryo is also a preferred testing model to adult fish because it is anticipated that early life stages feel less pain and distress than adult fish. Therefore, zebrafish early life stages have been proposed as an effective model for toxicity studies (reviewed in [5]) and test guidelines have been developed and validated using zebrafish embryos as model organism (Fish Embryo Acute Toxicity (FET), OECD test guideline 236) [6].

Thus far, a few studies have assessed the reproducibility of rodent and rabbit toxicity with zebrafish embryo toxicity testing (reviewed in [5]). Some of these studies focus on a few chemicals in one class of compound. For example, one study showed that zebrafish embryo results from four organotin compounds accurately reflected the general developmental and neuro-developmental toxicity effects found in rodents [7]. Another study showed that six 1,2,4-triazole compounds tested in zebrafish embryos mimicked the results of rodent testing [8]. A single laboratory study by Ali and colleagues showed that there was a strong correlation between zebrafish embryo LC50 values and rodent LD50s for a set of 60 different compounds [9]. Another single laboratory study investigated both toxicity and teratogenicity of 18 compounds and concluded that toxic responses in zebrafish are comparable to those in mice [10]. However, whether zebrafish toxicity data can be collated from several different studies and correlated to rodent data has not been reported. Here, we set out to analyze how metadata from zebrafish toxicity during early life stages relates to rodent and rabbit toxicity. We first ranked chemicals tested in zebrafish embryo and larvae based on toxicity and teratogenicity, and we developed a potential hazard index based on human exposure data. Next, we performed a correlation analysis of rodent and rabbit acute toxicity and developmental toxicity data with zebrafish developmental toxicity data. These correlation and ranking findings have implications for future standards and testing guidelines. The results can be used for prioritizing chemical testing in mammalian species with the ultimate goal to protect human health.

2. Methods

2.1. Zebrafish toxicity and lethality data

We utilized the dataset of 120 publications from Ducharme et al. (2013) [11] and added 77 additional published studies from the literature available via PubMed through June 1, 2013 (listed in Supplemental Table 1). Inclusion criteria were: Publications describing exposure to environmental or industrial chemicals in zebrafish; Treatment and scoring period from 0 to 7

days post fertilization (dpf); Presentation of a statistically significant effect after exposure to the test chemical that was different from unexposed or vehicle exposed fish. The exclusion criteria were: Toxicity data only reported for pharmaceuticals (to keep within the scope of this study); Publications with non-significant data. All of these data were categorized according to our previously established system [11] with the addition of more subcategories for statistical analysis. For the different endpoints we listed the Lowest Observed Adverse Developmental Effect Dose (LOADED). This value represents the lowest reported dose that had an adverse outcome for a certain endpoint if a range of concentrations was tested or the treatment dose used if a range was not reported, and it should not be equalized to Lowest Adverse Effect Level (LOAEL), which is a calculated number often used in toxicology, but which requires a full dose response experiment. For the chemicals from Padilla and colleagues [12] we used the AC10 value instead of the LOADED. We also listed the Lethal Concentration for 50% of the embryos (LC50) values. In publications in which it was not possible to calculate an LC50, we listed the lower dose closest to 50% lethality. If multiple studies examined the same chemical and broad endpoint, we used the lowest reported dose that had an adverse effect for our analysis. In total, the data include 600 chemicals analyzed at 19 aggregated developmental endpoints, three physical parameters, and the no observed adverse effect dose yielding 2695 unique data points. The aggregated endpoints were also subdivided into individual endpoints (e.g. Behavioral Responses were subdivided into spontaneous movement, touch response, swimming, and other) such that 78 zebrafish endpoint categories were evaluated. A list of the endpoints that were chosen and how they were clustered into broader categories is shown in Supplemental Table 2. The data can be downloaded from <http://cbl.uh.edu/Zebrafish>

2.2. Mammalian lethality data

Mouse, rat, and rabbit LC50 (inhalation) and LD50 (all other exposure routes) values were collected for this study from both ChemIDplus and the Hazardous Substances Data Bank (HSDB), available through TOXNET (<http://toxnet.nlm.nih.gov/index.html>). If there were discrepancies between the databases, the lowest value was used. All mammalian acute toxicity data was converted to both mg/kg-day and M/day, assuming that 1 kg body weight was equivalent to 1 L.

2.3. Human exposure data

The theoretical daily dose (TDD) for humans from the 2011 Substance Priority List was downloaded from the ATSDR web-site (<http://www.atsdr.cdc.gov/SPL/resources/index.html>). It lists the substances that are most commonly found at facilities on the National Priorities List (NPL) and which are determined to pose the most significant potential threat to human health due to their known or suspected toxicity and potential for human exposure at these NPL sites. The reference doses were downloaded from the US EPA's IRIS database (<http://www.epa.gov/iris/standal.html>). All data was converted to M/day and mg/kg-day, assuming that 1 kg equals 1 L. Wetmore and colleagues gathered human exposure data from federal documents [13]. These exposure values were found in Supplementary Table 7 of Wetmore et al. [13], and converted to M/day and mg/kg-day, assuming that 1 kg equals 1 L.

2.4. Statistical analysis

Statistical analyses were performed as previously described [11]. Briefly, non-parametric Spearman rank correlation analysis was performed to identify the association between pairs of endpoints, where compounds formed the sample size. For outcome (affected endpoint) correlations, we required that each pair of outcomes have an overlap of at least 6 different chemicals in order to allow the p -value for the Spearman's rho to be equal or less than 0.05 [14]. All Spearman analyses were performed on consistent units of measure (molar values compared to molar, mg/kg-day compared to mg/kg-day).

3. Results

3.1. Ranking of chemicals based on zebrafish toxicity

We collected zebrafish developmental toxicity and lethality values for 600 chemicals from studies published in PubMed (www.ncbi.nlm.nih.gov/pubmed). First, we recorded the Lowest Observed Adverse Developmental Effect Dose (LOADED) of a chemical for any endpoint (listed in Supplemental Table 2) and the Lethal Concentration at which 50% of the embryos died (LC50) scored at different time points. We next set out to rank the chemicals based on zebrafish toxicity. The ranking based on LOADED is shown in Table 1 for the top 15 most toxic chemicals, and for 443 chemicals in Supplementary Table 3. From this ranking we conclude 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 1,2,3,7,8-pentachlorodibenzo-p-dioxin, retinoic acid, and chlorpyrifos were the most toxic chemicals investigated in zebrafish so far, while sodium thiosulfate, saccharin, paraoxon-methyl, and acetone were the least toxic.

We next ranked the chemicals based on teratogenicity. We here define the teratogenicity as the capability of an agent to produce fetal malformation relative to lethality. A teratogen ratio was calculated by dividing the LC50 value with the LOADED value (excluding lethality) (LC50/LOADED), as described previously [11]. This ranking included 148 chemicals that had both a LOADED and LC50 value. The top 15 most teratogenic chemicals are shown in Table 2, and the full list is shown in Supplementary Table 4. This list clusters the chemicals into three distinct groups: highly teratogenic (1000 and above), mid-level (100–1000), and low-level (below 100) teratogens. We conclude that potassium perchlorate, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), diethyl dithiocarbamate disulfide (dithiocarbamate), carbon disulfide, and benzo(a)pyrene ranked as the most potent teratogens, while cadmium chloride, 2,6-dihydroxyphthalene, fenvalerate, ethanol, and acrylamide were found by the end of the teratogen ranking list based on assessments in zebrafish.

3.2. Correlation of zebrafish toxicity values with mammalian acute toxicity values

We have previously investigated correlations between chemically induced malformations within the zebrafish model [11]. We now set out to examine whether zebrafish developmental toxicity correlates to acute toxicity in rats, mice, and rabbits. We choose to compare the zebrafish toxicity to acute mammalian toxicity because the exposures times in zebrafish selected for this study were short, often just in the form of a single exposure. The exposure routes in zebrafish include dermal exposure, exposure through the gills and

exposure via ingestion at fish stages after mouth opening. The exposure routes for the mammalian acute toxicity data included inhalation, oral, and dermal exposures, as well as direct injection of the compound. For the correlations, we required that each pair of outcomes have an overlap of at least 6 different chemicals.

United States Environmental Protection Agency's (US EPA's) "Acute Inhalation Toxicity" guidelines require the use of the rat for acute inhalation studies, unless there is a justifiable reason for using another mammalian species [15]. We found that the zebrafish LC50 that were scored at 96 h post fertilization (hpf) correlated with rat LC50 inhalation values (Spearman $\rho = 0.87$; $p = 0.002$; $N = 10$; $R^2 = 0.74$) (Fig. 1a). Less than six overlapping chemicals were found for either rabbit or mouse inhalation LC50s with zebrafish LC50s, and thus a correlation could not be determined. However, when aggregating all of the inhalation LC50 values from the three rat, mouse, and rabbit species, a similar positive correlation was apparent with zebrafish LC50s at 96 hpf ($\rho = 0.85$; $p = 0.0008$; $N = 12$) (Table 3). When dividing the rodent and rabbit inhalation values with the zebrafish LC50 values for each chemical, we found that although the ratio varied between different chemicals, the zebrafish was on average 180 times more sensitive than the mammalian system for inhalation (Supplemental Fig. 1). This finding is worth noting for calculating species to species conversion factors.

US EPA's "Acute Oral Toxicity" guidelines similarly suggest the use of the rat for acute oral toxicity [16]. We did not find that zebrafish LC50 values correlated to rodent or rabbit oral toxicity LD50s (ρ values ranged between 0.31 and 0.61). However, we did observe that the LOADED values for alterations in early spontaneous movement in zebrafish correlated to both mouse and rat oral LD50 doses (mouse: $\rho = 0.86$; $p = 0.02$; $N = 7$; rat: $\rho = 0.85$; $p = 0.003$; $N = 10$; $R^2 = 0.76$) (Fig. 1b and Table 4). Rabbit LD50 values correlated with LOADED values for alterations in zebrafish internal organ development ($\rho = 1$; $p = 0.003$; $N = 6$) (Table 4).

US EPA's "Acute Dermal Toxicity" guidelines prefer the use of rabbits, although rats or guinea pigs are also allowable for investigating exposures through the dermal route [17]. Rabbit dermal LD50 values correlated with the LOADED values for decreased touch response and decreased heart rate in zebrafish (touch response: $\rho = 0.82$, $p = 0.034$; $N = 7$; $R^2 = 0.91$; heart rate: $\rho = 0.85$; $p = 0.003$; $N = 10$; $R^2 = 0.91$) (Fig. 1c and Table 4). We did not find any zebrafish endpoints that correlated with rat or mouse dermal exposure LD50 values, although many endpoints (72 out of 80 endpoints for mouse and 57 out of 80 for rat) did not have six overlapping chemicals, and therefore could not be investigated.

While inhalation, oral, and dermal acute toxicity testing is the starting point for US EPA's testing guidelines, other lethal doses for rodents have been collected over time. Many of these additional LD50 values also correlated with individual zebrafish outcomes. The LD50 for the aggregation of all three mammalian species' subcutaneous injections correlated with the LC50 for zebrafish at 120 hpf ($\rho = 0.85$; $p = 0.004$; $N = 12$). Mouse LD50s for intraperitoneal (IP) injections also correlated with the zebrafish LC50 at 120 hpf ($\rho = 0.84$; $p = 3.528E-06$; $N = 20$) (Table 3). Rabbit LD50s for IP injections correlated with the LOADED values for pericardial edema ($\rho = 0.94$; $p = 0.0167$; $N = 6$) in zebrafish, among

other outcomes (Table 4). LD50s for intravenous (IV) injections positively correlated with zebrafish LOADED values for pericardial edema for both mouse ($\rho = 0.94$; $p = 0.001$; $N = 12$) and rat ($\rho = 0.95$; $p = 0.001$; $N = 8$). In addition, mouse IV LD50 values correlated with alterations in LOADED values for zebrafish touch response ($\rho = 0.86$; $p = 0.0238$; $N = 7$), ear development ($\rho = 0.89$; $p = 0.0123$; $N = 7$), and general early development defects ($\rho = 0.80$; $p = 0.0211$; $N = 8$). Rabbit IV LD50 values correlated with zebrafish LOADED values for general behavioral changes ($\rho = 0.89$; $p = 0.00605$; $N = 8$). Rat and mouse IV LD50 values also correlated with alterations in zebrafish LOADED values for somite development (rat: $\rho = 0.86$; $p = 0.0238$; $N = 7$; mouse: $\rho = 0.85$; $p = 0.00607$; $N = 9$). We also divided the correlating rodent and rabbit LD50 values with the zebrafish LC50 values. The resulting ratios varied substantially; on average the zebrafish system was about 100 times more sensitive than rodent and rabbit injection LD50s, but the mean ratio was 1 (Supplemental Fig. 1). Thus, the difference in sensitivity varied by compound and endpoint.

Taken together, we found correlations between several endpoints of zebrafish toxicity with rabbit, rat, or mouse LD50s for overlapping chemicals. In particular, zebrafish LC50 values and LOADED values for endpoints of the behavior category correlated to the rodent and rabbit LD50 data.

3.3. Correlation of zebrafish embryo toxicity endpoints to rat and rabbit developmental toxicity

We utilized the data from ToxRefDB (<http://actor.epa.gov/actor/faces/ToxCastDB/DataCollection.jsp>) to compare rabbit and rat developmental endpoints to zebrafish developmental endpoints. Unfortunately, 75.5% of the possible pairings did not have at least six overlapping chemicals, and thus could not be analyzed. The lowest effect levels (LELs) for rabbit and rat prenatal loss (defined as pregnancy loss or maternal wastage (including implantations loss) and fetal death impacting litter size) correlated with each other ($\rho = 0.93$, $p = 4.1E-21$, $N = 47$) (Table 5). However, the LEL for developmental abnormalities did not significantly correlate between the species or with prenatal loss. Strikingly, zebrafish LOADED values for altered hatching rate positively correlated with rabbit LELs for prenatal loss ($\rho = 0.93$, $p = 0.002$, $N = 8$) (Table 5). The LOADED values for aggregated cardiovascular category ($\rho = 0.95$, $p = 0.0001$, $N = 10$) as well as the individual cardiovascular categories of pericardial edema ($\rho = 0.83$, $p = 0.015$, $N = 8$) and decreased heart rate ($\rho = 0.89$, $p = 0.033$, $N = 6$) also correlated with the LELs for rabbit prenatal loss (Table 5). These correlations indicate that despite the limited data overlap, correlations between mammalian and zebrafish developmental toxicity are apparent and warrant further investigation.

3.4. Hazard index based on human exposure and LOADED

Human exposure doses have been collected and were found in two separate databases. Wetmore and colleagues published human exposure values gathered from federal documents [13]. In addition, the Agency for Toxic Substances and Disease Registry (ATSDR) publishes the theoretical daily dose (TDD) for humans for chemicals on the Substance Priority List. 157 out of the 444 chemicals with a zebrafish LOADED also had a human exposure value in one of the databases. To incorporate exposure data into our ranking of chemicals, we

calculated a hazard index by dividing the human exposure level (as M/day, with the assumption that 1 kg human equals 1 L) with the zebrafish LOADED. The hazard index should be viewed as a value to guide prioritizing chemicals for further studies in rodents, and not to predict a human health impact, since the correlation of zebrafish toxicity and human health is not known. Nevertheless, a high hazard index for a chemical indicates that humans could be exposed to levels of this chemical above or close to a level where it is toxic in zebrafish. We found that relatively few chemicals had a high hazard index; 14 chemicals had a hazard index above 1.0 (Supplemental Table 5). Comparing the 111 chemicals found on the Wetmore list suggests that fludioxonil, tefluthrin and pyridaben had a high hazard index (Supplemental Table 5). In contrast, fenamiphos, tri-allate, and disulfoton had the lowest hazard index (Supplemental Table 5). The toxic exposure on NPL sites is higher than the general population exposure data gathered by Wetmore since NPL sites are highly contaminated sites. The hazard index for the 55 overlapping chemicals found on NPL sites suggested that benzene, diniconazole, benzo(a)pyrene, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), atrazine, and pentachlorophenol (PCP) had the highest hazard index (Table 6 and Supplementary Table 5). When merging the Wetmore exposure data and the NPL TDD for the chemicals (selecting the highest hazard index of overlapping chemicals), we obtained values for 165 chemicals, but the ranking of the top 13 chemicals did not deviate from the list generated using only the Superfund site data.

4. Discussion

We here present ways of ranking chemicals based on toxicity, as determined by the LOADED value, and teratogenicity (LC50/LOADED) in zebrafish early life stages. In addition, we propose a potential hazard index (human exposure/LOADED) as a tool to prioritize chemicals for further investigations in higher vertebrates. These three assessments provide diverse methodologies to assess the potential hazards of chemicals with available data. For example, TCDD ranks in the top four of all three lists and should be considered a high priority chemical for environmental cleanup and further evaluation. The polycyclic aromatic hydrocarbon benzo(a)pyrene is in the top six of all three lists. TCDD and benzo(a)pyrene are produced in residential wood burning, waste incineration, and as a bi-product in industry. The insecticide chlorpyrifos ranks in the top nine for the three lists. Chlorpyrifos is a highly produced insecticide used in agriculture for cotton, corn, almonds and fruit trees, and is detected in both streams and ground water [18]. Interestingly, TCDD, benzo(a)pyrene and chlorpyrifos have all been shown to target the aryl hydrocarbon receptor [19,20]. For these three compounds, the daily human exposure concentrations are predicted to be 5–60 times higher than what induces toxicity in zebrafish embryos (often by a single exposure). Benzene, highly produced in the petrochemical industry, had the highest hazard index, and was predicted to have a human exposure concentration approximately 5000 times higher than what caused a toxic effect in zebrafish. The hazard index that we present was calculated for 157 chemicals that had both zebrafish toxicity and human exposure data. However, the use of a potential hazard index would be greatly enhanced with increased collection of actual human exposure data instead of predicted exposure levels. While a limited dataset does exist [21] and was incorporated by Wetmore and colleagues [22], the

original NHANES dataset only included 10 overlapping compounds with our zebrafish toxicity list.

Vulnerable populations for chemical exposure are the developing fetus, newborn babies, and children, as exposures at these early time points might lead to developmental defects. We and others have previously shown that a teratogen ratio or index calculated from zebrafish toxicity and lethality data to a large extent correctly ranks known mammalian teratogens [11,23,24]. We here determined that out of 148 chemicals, 11 chemicals were highly teratogenic in zebrafish with a teratogen ratio above 1000. This value demonstrates the chemicals cause a defect in zebrafish at a minimum of 1000 times lower concentrations than the LC50. The chemicals with high teratogen ratios include potassium perchlorate (teratogen ratio 251016.67), TCDD (teratogen ratio 167424.92), and diethyl dithiocarbamate disulfide (dithiocarbamate) (teratogen ratio 10,000). Potassium perchlorate, used in fireworks, explosives, and also as a drug to treat hyperthyroidism, is detected as a contaminant in drinking water [25]. The wide-spread pollutant TCDD has relatively low human exposure levels. However, the zebrafish effect dose is extremely low, as well, yielding a very high teratogen ratio. The teratogen ratios here are calculated based on zebrafish embryo toxicity, and the correlation between zebrafish and human developmental toxicity is not known. But since several developmental pathways are conserved between lower and higher vertebrates, the zebrafish teratogen ratio might serve as an indicator that there is a need to thoroughly assess the teratogenic effects of these high ranking compounds in higher vertebrates. Bisphenol A (BPA), which we previously reported had a teratogen ratio of 8.9 [11], advanced on the teratogen ranking because of a number of new studies showing toxic effects at very low concentrations (see Supplemental Table I), and is now the 9th most teratogenic compound on our list (teratogen ratio 2000). Although human exposure levels for BPA were not included in the Wetmore and NPL exposure data, it is known that infants have up to 11 times higher levels of BPA in their blood than adults [26,27]. Using this infant exposure range of 1–19.4 nM would give a maximal hazard index of 1.94.

We have previously reported how different toxicity outcomes (affected endpoints) within the zebrafish model correlate to each other [11]. Now we further sought to ascertain if zebrafish toxicity and lethality values correlated to rodent and rabbit acute toxicity and lethality values. If it did, the correlating zebrafish endpoints could be further tested and evaluated for developing an endpoint-specific zebrafish embryo test, which could be part of a strategy to reduce or replace mammalian testing. Although many zebrafish endpoints did not correlate to available mammalian acute toxicity data, we found that some zebrafish outcomes do provide an accurate reflection of rodent and rabbit toxicity. The zebrafish could be used to accurately predict relative acute toxicity as a surrogate for the trifecta: rat inhalation, rabbit dermal, and rat oral exposures through correlations to zebrafish LC50 values and LOADED values for behavioral endpoints (Table 7). However, these correlations were detected with a relatively low number of chemicals, and more data will be required to further evaluate potential correlations between zebrafish and rodent/rabbit toxicities. Mouse inhalation, mouse oral, rat inhalation, and rat oral lethality values correlated to LOADED and LC50 values in zebrafish that could be assessed within 96 hpf. Certain zebrafish endpoints that showed correlations to rodent and rabbit toxicity, such as spontaneous movement, could be assessed even earlier at 24 hpf. Thus, using zebrafish as a model for toxicity testing would

decrease testing times and costs substantially, in comparison to rodent and rabbit testing with the requirement of testing at age 8–12 weeks (rats) or at least 12 weeks (rabbits) and then observing the animals for a minimum of 14 days. Also, the zebrafish embryo is very small and zebrafish have a high fecundity, making them amendable for high throughput toxicity screening.

The rabbit dermal exposure and rat oral exposure LD50s correlated with LOADED values for subcategories of zebrafish behavior (spontaneous movement and touch response). Zebrafish behavioral outcomes require a coordinated response of the nervous and muscle systems in order to initiate spontaneous movement or respond to touch. Because these outcomes require multiple system inputs, they may be an accurate reflection of even subtle impacts on the individual systems through their aggregation. In addition, there are several devices available to track zebrafish behavior, which in an automated setup would facilitate high throughput toxicity testing. This testing regime would be efficient for pre-screening chemicals for toxicity, and could serve as a basis for prioritization of chemicals for further testing on other zebrafish toxicity endpoints or in higher vertebrates.

One of the major caveats with our study is the lack of overlapping data between mammalian and zebrafish outcomes. In particular, large data gaps exist between developmental rat and rabbit endpoints and zebrafish toxicity. Nevertheless, we can begin to draw correlations based on our data set. In particular, some rabbit developmental toxicity outcomes were predicted accurately by zebrafish toxicity. The lowest effect levels for rabbit prenatal loss correlated with the LOADED values for altered hatching rate, decreased heart rate, and pericardial edema in the zebrafish embryos. The assessment of rabbit prenatal loss requires a minimum of 80 rabbits with implantation sites with daily treatment through parturition per chemical tested [28]. If the relationship between zebrafish endpoints and rabbit prenatal loss is supported in future studies, a significant reduction of testing time and mammalian animal lives would be possible through utilizing the zebrafish model system. Therefore, the field would benefit from a concerted initiative to increase the number of overlapping chemicals in order to ascertain if these correlations are substantiated with larger data sets.

If the correlation between relative chemical toxicity between zebrafish and mammals is supported with further analysis with additional overlapping chemicals, one of the challenges will be to determine a conversion factor to apply to the zebrafish values in order to obtain a mammalian or human equivalency factor. The current standard is to scale rodent body weight (BW) to the $3/4$ power ($BW^{3/4}$) followed by an interspecies uncertainty conversion factor of 10 to rodent values when determining the equivalent human dose [29]. However, as shown in Supplemental Fig. 1, the conversion factor between zebrafish and rodent and rabbit lethal doses spans a wide range. Thus, a dedicated effort to determine the appropriate conversion factor would need to be instituted. For several chemicals, the zebrafish LC50 value was lower than the corresponding rabbit/rodent LD50s. Similarly, Parng and colleagues have shown that the log median lethal dose in zebrafish is generally lower than that in mammals [10]. It should be noted that in a majority of zebrafish toxicity studies, the chemical concentrations refer to water concentrations and not the actual concentration in the fish. Furthermore, the different exposure routes in rodents and zebrafish likely cause different uptake of the chemicals. Thus, chemical properties and uptake as defined by

QSARs should be taken into account to determine conversion factors between fish and rodents or human.

In conclusion, zebrafish embryo toxicity testing shows promise in serving as an initial screening method and possibly as a surrogate for mammalian toxicity testing. As nations seek to reduce mammalian animal testing, the zebrafish embryo is emerging as a viable alternative to prioritize compounds for developmental toxicity testing in higher vertebrates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.reprotox.2014.09.005>.

Fig. 1.

Correlation between selected rodent and rabbit lethal dose/concentrations and zebrafish embryo toxicity outcomes. (a) The LC50 for zebrafish at 96 hpf was plotted against the LC50 rat inhalation values. The R^2 value for the line is 0.74. (b) The concentration of a chemical causing an alteration in zebrafish spontaneous movement was plotted against the LD50 oral rat value. The R^2 value for the line is 0.76. (c) The concentrations of a chemical causing a change in zebrafish touch response or decreased heart rate were plotted against the LD50 rabbit dermal value. The R^2 for the both outcomes is 0.91.

Table 1

Ranking of chemicals by the lowest observed adverse effect dose (LOADED) for zebrafish embryo toxicity.

Compound	CAS	LOADED (M)	Endpoint ^a
2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)	1746-01-6	1.55E-13	Heart rate decreased Pericardial edema Yolk sac edema
1,2,3,7,8-Pentachlorodibenzo-p-dioxin	40321-76-4	1.12E-12	Pericardial edema
Retinoic acid	302-79-4	2.13E-10	Curvature
Chlorpyrifos	2921-88-2	2.85E-10	Behavior
Platinum chloride	10025-65-7	3.76E-10	Heart rate decreased
Benzo(a)pyrene	50-32-8	9.51E-10	Hatching rate altered
Triphenyltin acetate (TPTA)	900-95-8	1.22E-09	Hatching rate altered
Benzene	71-43-2	1.29E-09	Blood flow altered
Aldicarb	116-06-3	1.36E-09	AC10/EC10
Flumetralin	62924-70-3	1.36E-09	AC10/EC10
Flumiclorac-pentyl	87546-18-7	1.36E-09	AC10/EC10
Thiram	137-26-8	1.36E-09	AC10/EC10
Milbemectin (mixture)	51596-11-3	1.88E-09	AC10/EC10
Endosulfan I	959-98-8	2.45E-09	Touch response
Abamectin	71751-41-2	2.68E-09	AC10/EC10

^aEndpoint column denotes those endpoints that were affected at the LOADED value.

Table 2

Ranking of chemicals by zebrafish teratogen ratio (LC50/LOADED).

Compound	CAS	Teratogen ratio
Potassium perchlorate (KClO ₄)	7778-74-7	251016.67
2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)	1746-01-6	167424.92
Diethyl dithiocarbamate disulfide (dithiocarbamate)	4384-82-1	10000.00
Carbon disulfide	75-15-0	7766.99
Benzo(a)pyrene	50-32-8	5361.57
Chlorpyrifos	2921-88-2	3505.86
Dimethyldithiocarbamate	128-04-1	2325.00
N,N-Dimethylformamide (DMF)	68-12-2	2211.09
Bisphenol A	80-05-7	2000.00
Parathion	56-38-2	1000.00
Resorcinol (RES)	108-46-3	961.00
4-Chloroaniline	106-47-8	880.00
Boric acid	10043-35-3	762.14
Retinoic acid	302-79-4	629.11
Benzene	71-43-2	496.22

Table 3

Spearman correlations between rodent and rabbit lethality values (M) and zebrafish LC50s (M) scored at different time points.

	ZF LC50 24 hpf	ZF LC50 48 hpf	ZF LC50 72 hpf	ZF LC50 96 hpf	ZF LC50 120 hpf	ZF LC50 144 hpf	ZF LC50 168 hpf
Mouse inhalation LC50			<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6
Mouse intraperitoneal LD50	0.55 ^{**} (<i>n</i> = 34)	0.52 ^{**} (<i>n</i> = 26)	0.61 ^{**} (<i>n</i> = 23)		0.84^{**} (<i>n</i> = 20)	0.61 ^{**} (<i>n</i> = 23)	
Mouse oral LD50	0.55 ^{**} (<i>n</i> = 35)	0.45 [*] (<i>n</i> = 27)	0.39 [*] (<i>n</i> = 28)		0.68 ^{**} (<i>n</i> = 15)	0.58 ^{**} (<i>n</i> = 25)	
Mouse dermal LD50	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6
Rabbit inhalation LC50	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6
Rabbit intraperitoneal LD50	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6
Rabbit oral LD50				0.73 ^{**} (<i>n</i> = 17)	<i>n</i> < 6		
Rabbit dermal LD50			<i>n</i> < 6		<i>n</i> < 6		<i>n</i> < 6
Rat inhalation LC50			<i>n</i> < 6	0.87^{**} (<i>n</i> = 10)			<i>n</i> < 6
Rat intraperitoneal LD50		0.65 ^{**} (<i>n</i> = 21)					
Rat oral LD50	0.52 ^{**} (<i>n</i> = 38)	0.48 ^{**} (<i>n</i> = 32)	0.57 ^{**} (<i>n</i> = 29)		0.59 ^{**} (<i>n</i> = 24)	0.62 ^{**} (<i>n</i> = 29)	
Rat dermal LD50		<i>n</i> < 6	<i>n</i> < 6		<i>n</i> < 6		<i>n</i> < 6
Rodent and rabbit minimum inhalation LC50				0.85^{**} (<i>n</i> = 12)			<i>n</i> < 6

Bold text indicates positive correlation with $\rho > 0.79$. “*n*” refers to the number of overlapping chemicals between endpoints. No value indicates that the intersection was not statistically significant ($P > 0.05$).

* Statistical significance $P < 0.05$.

** Statistical significance $P < 0.01$.

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Table 4

Spearman correlations between rodent and rabbit lethality values (M) and selected zebrafish embryo toxicity endpoints (M).

	ZF behavior/spontaneous movement	ZF behavior/touch response	ZF behavior/swimming	ZF behavior/all	ZF cardiovascular/ heart rate decreased	ZF cardiovascular/pericardial edema	ZF cardiovascular/all	ZF internal organs/all
Mouse inhalation LC50	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6		0.59 [*] (<i>n</i> = 13)	<i>n</i> < 6
Mouse intraperitoneal LD50	<i>n</i> < 6	0.72 [*] (<i>n</i> = 11)	0.49 [*] (<i>n</i> = 17)	0.55 ^{**} (<i>n</i> = 29)	0.55 ^{**} (<i>n</i> = 24)		0.46 ^{**} (<i>n</i> = 50)	<i>n</i> < 6
Mouse oral LD50	0.86[*] (<i>n</i> = 7)		0.58 [*] (<i>n</i> = 17)	0.48 [*] (<i>n</i> = 32)	0.48 ^{**} (<i>n</i> = 32)		0.46 ^{**} (<i>n</i> = 55)	0.79 ^{**} (<i>n</i> = 15)
Mouse dermal LD50	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6			<i>n</i> < 6
Rabbit inhalation LC50	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6		<i>n</i> < 6	<i>n</i> < 6
Rabbit intraperitoneal LD50	<i>n</i> < 6	<i>n</i> < 6	<i>n</i> < 6	0.9^{**} (<i>n</i> = 9)	<i>n</i> < 6		0.95^{**} (<i>n</i> = 8)	<i>n</i> < 6
Rabbit oral LD50	<i>n</i> < 6	0.7 [*] (<i>n</i> = 10)			0.65 ^{**} (<i>n</i> = 17)		0.46 ^{**} (<i>n</i> = 28)	1.0^{**} (<i>n</i> = 6)
Rabbit dermal LD50	<i>n</i> < 6	0.82[*] (<i>n</i> = 7)			0.85^{**} (<i>n</i> = 10)			<i>n</i> < 6
Rat inhalation LC50	<i>n</i> < 6							
Rat intraperitoneal LD50	<i>n</i> < 6			0.41 [*] (<i>n</i> = 27)				
Rat oral LD50	0.85^{**} (<i>n</i> = 10)	0.66 ^{**} (<i>n</i> = 14)	0.45 ^{**} (<i>n</i> = 25)	0.53 ^{**} (<i>n</i> = 42)	0.49 ^{**} (<i>n</i> = 41)		0.49 ^{**} (<i>n</i> = 71)	0.65 ^{**} (<i>n</i> = 21)
Rat dermal LD50	<i>n</i> < 6	<i>n</i> < 6						<i>n</i> < 6

Bold text indicates positive correlation with rho > 0.79, “*n*” refers to the number of overlapping chemicals between endpoints. No value indicates that the intersection was not statistically significant (*P* > 0.05).

^{*} Statistical significance *P* < 0.05.

^{**} Statistical significance *P* < 0.01.

Table 5

Rodent and rabbit early developmental defects (M) correlations with zebrafish developmental outcomes (M).

	Rabbit prenatal loss	Rat prenatal loss	Rat viability PND4	Rabbit DEV LEL	Rat DEV LEL
Rabbit prenatal loss	1				
Rat prenatal loss	0.93 ^{***} (<i>n</i> = 47)	1			
Rat viability PND4			1		
Rabbit DEV LEL			0.72 ^{***} (<i>n</i> = 25)	1	
Rat DEV LEL			0.55 ^{***} (<i>n</i> = 36)	0.42 ^{***} (<i>n</i> = 64)	1
ZF hatching rate altered	0.93 ^{***} (<i>n</i> = 8)	<i>n</i> < 6			
ZF heart rate decreased	0.89 [*] (<i>n</i> = 6)	<i>n</i> < 6			
ZF pericardial edema	0.83 [*] (<i>n</i> = 8)	<i>n</i> < 6	-0.74 [*] (<i>n</i> = 8)		

Bold text indicates positive correlation with $\rho > 0.79$. “*n*” refers to the number of overlapping chemicals between endpoints. No value indicates that the intersection was not statistically significant ($P > 0.05$). 1 marks the comparison between the same categories.

^{*} Statistical significance $P < 0.05$.

^{***} Statistical significance $P < 0.01$.

Table 6

Ranking of chemicals by potential hazard index (human exposure value/LOADED).

Compound	Wetmore hazard index	TDD* hazard index	Combined** hazard index
Benzene		4.98E+03	4.98E+03
Diniconazole		3.37E+02	3.37E+02
Benzo(a)pyrene		1.52E+02	1.52E+02
2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)		6.05E+01	6.05E+01
Atrazine		5.36E+01	5.36E+01
Pentachlorophenol (PCP)		8.21E+00	8.21E+00
Endosulfan I		6.22E+00	6.22E+00
4-Chloroaniline		5.12E+00	5.12E+00
Chlorpyrifos		4.90E+00	4.90E+00
Tributyltin		4.44E+00	4.44E+00
Hexabromocyclododecane		3.32E+00	3.32E+00
Silver		2.69E+00	2.69E+00
Parathion		2.16E+00	2.16E+00
Fludioxonil	1.28E+00		1.28E+00
Cadmium		4.03E-01	4.03E-01

* TDD = Theoretical Daily Dose from National Priority List Superfund Sites.

** Minimum human exposure of aggregated exposure data from TDD and Wetmore (Wetmore et al., 2013).

Table 7

Zebrafish embryo endpoint with highest correlation to rodent and rabbit acute toxicity.

Rodent and rabbit acute toxicity exposure route	Zebrafish embryo endpoint
Mouse inhalation LC50	LC50 at 96 hpf
Rat inhalation LC50	LC50 at 96 hpf
Mouse oral LD50	Spontaneous movement at 24 hpf
Rat oral LD50	Spontaneous movement at 24 hpf
Rabbit dermal LD50	Touch response at 120 hpf
Mouse subcutaneous injection LD50	LC50 at 120 hpf
Rat subcutaneous injection LD50	LC50 at 120 hpf
Rabbit subcutaneous injection LD50	LC50 at 120 hpf